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A REVIEW OF THE TYPES OF VENTILATION AND THEIR NEURAL CONTROL IN AESHNID LARVAE

P.J. MILL & R.S. PICKARD
Department of Pure and Applied Zoology, University of Leeds, Leeds, LS2 9JT, England.

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Aeshnid larvae respire through tracheal gills. These are located in a modified region of the hind gut, the branchial chamber, and are ventilated by muscular movements which effect a flow of water into and out of the anus. Normal ventilation is a rhythmic process, with expiration occurring first. The nature and nervous control of this and other forms of ventilation, including jet-propulsive swimming, are analysed in terms of morphology, mechanics and neurophysiology.

It has been known for some time that anisopteran dragonfly larvae respire through tracheal gills located in a modified posterior region of the alimentary canal known as the branchial chamber (AMANS, 1881; MATULA, 1911; TILLYARD, 1917; WALLENGREN, 1913, 1914a, 1914b). However, TONNER (1936) was the first to describe different types of ventilatory movements. He distinguished normal, gulping and chewing ventilation, and in addition noted that the jet-propulsive mechanism of swimming, in which the larva ejects a current of water from its anus, would also subserve respiration. The various types of ventilation have been further analysed in recent years by HUGHES & MILL (1966), MILL (1970), MILL & HUGHES (1966), MILL & PICKARD (1972) and PICKARD & MILL (1972).

Furthermore, a great deal is known about the abdominal musculature (WALLENGREN, 1914; WHEDON, 1919; STEINER, 1929; MILL, 1965) and its innervation (ROGOSINA, 1928; MILL, 1965).

For studying the innervation of the muscles vital staining with methylene blue has generally been used (ZAWARZIN, 1911; ROGOSINA, 1928; MILL, 1965). As far as the mechanical and physiological aspects are concerned various techniques have been applied. Sternal movements have been recorded using a phototransistor (OCP 71) and a horizontal beam of light (HUGHES & MILL, 1966). In this last study pressure recordings were obtained from the branchial chamber and vestibule by inserting a fine hypodermic needle through the anus and attaching the other end to a Hansen condenser manometer. Movements of the anal valve and sternal movements have been photographed with a cine camera at speeds up to 64 frames/sec (MILL & PICKARD, 1972). Extracellular recordings from nerves and muscles have been obtained in one of two ways. In dissected preparations platinum wire electrodes were used (MILL & HUGHES, 1966) while in chronic preparations insulated copper wire electrodes were sealed in place with their ends in various muscles (PICKARD & MILL, 1972).

NORMAL VENTILATION

Normal ventilation usually occurs at frequencies of 25-50/min. in both *Aeshna* and *Anax* (HUGHES & MILL, 1966). It involves two distinct phases of approximately equal duration, expiration and inspiration, with expiration always

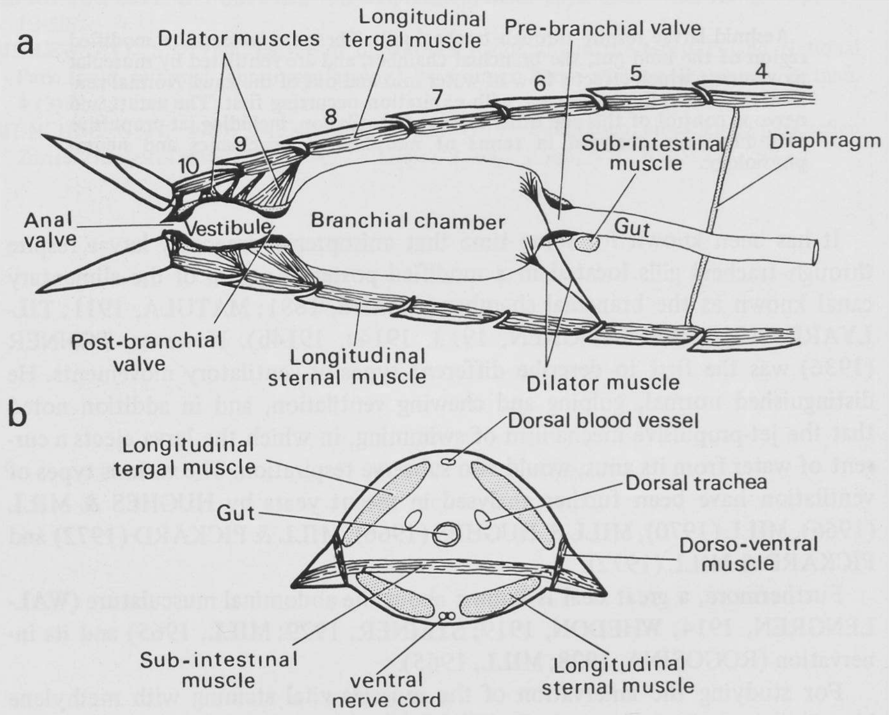

Fig. 1. *Anax*. Diagrams showing the principal structures concerned with ventilation in the anisopteran larva: (a) longitudinal, through the hind end of the abdomen; (b) transverse, through the anterior part of the sixth abdominal segment. (From MILL & PICKARD, 1972).

occurring first. The principal muscles involved in expiration are the paired respiratory dorso-ventral muscles (rdv's) of the posterior abdominal segments and probably also the intrinsic muscles of the vestibule (a muscular region of the hind-gut between the branchial chamber and the anus (Fig. 1). Each respiratory dorso-ventral muscle is innervated by a branch from the corresponding second segmental nerve (MILL, 1965) (Fig. 2).

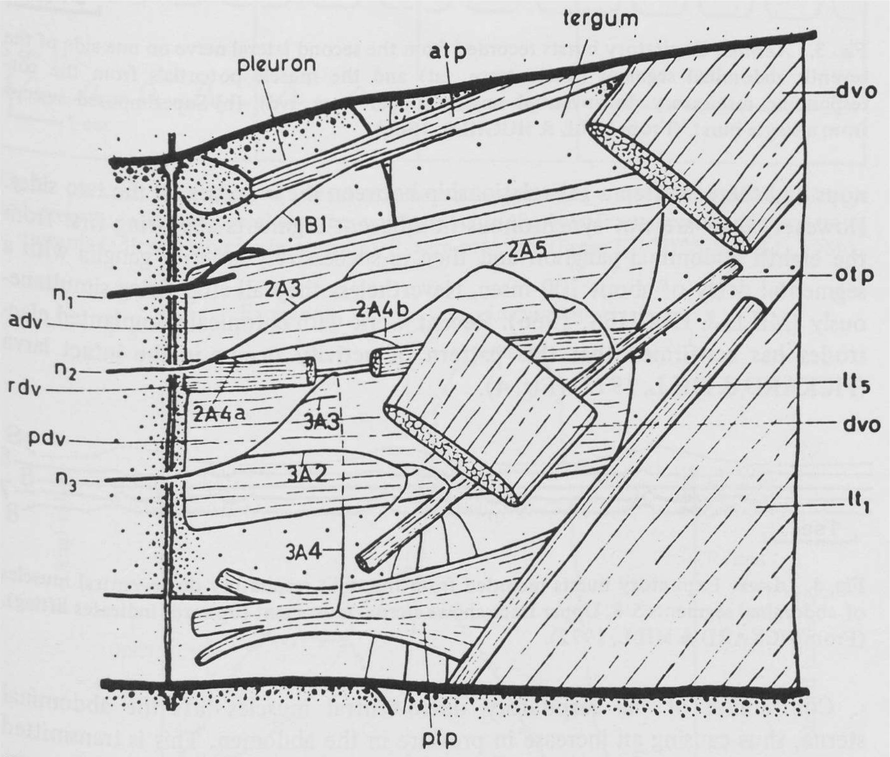

Fig. 2. *Aeshna.* Diagram illustrating the muscles in the pleural region of the right side of the fifth abdominal segment and their innervation. adv, anterior dorso-ventral muscle; atp, anterior tergo-pleural muscle; dvo, dorso-ventral oblique muscle; lt$_1$, lt$_5$, longitudinal tergal muscles; n$_1$-n$_3$, first-third lateral nerves; otp, oblique tergo-pleural muscle; pdv, posterior dorso-ventral muscle; rdv, respiratory dorso-ventral muscle; 1B1, etc. refer to the number of the nerve branch. (From MILL, 1965).

Rhythmic bursts of activity have been recorded from the second segmental nerves, the action potentials in one of the units demonstrating a 1:1 relationship with muscle potentials in the respiratory dorso-ventral muscles (MILL & HUGHES, 1966; MILL, 1970). (Fig. 3). Each expiratory burst is characterised by a steady build-up in the frequency of potentials followed by an abrupt cessation of activity. The bursts on opposite sides of the same segment are synchro-

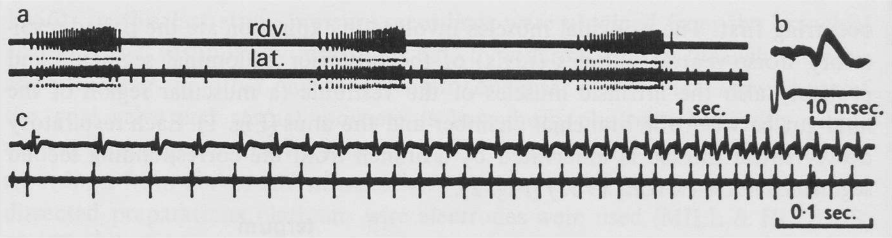

Fig. 3. *Aeshna.* Expiratory bursts recorded from the second lateral nerve on one side of the seventh abdominal segment (lower trace, lat) and the muscle potentials from the corresponding respiratory dorso-ventral muscle (upper trace, rvd). (b) Superimposed sweeps from a single burst. (From MILL & HUGHES, 1966).

nous and there is often a 1:1 relationship between the impulses on the two sides. However, they are not synchronous in adjacent segments appearing first from the eighth abdominal ganglion and then in successively anterior ganglia with a segmental delay of about 100 msec. Nevertheless they all stop firing simultaneously (MILL & HUGHES, 1966). Recent work with chronically implanted electrodes has confirmed that this pattern of activity occurs in the intact larva (PICKARD & MILL, 1972) (Fig. 4).

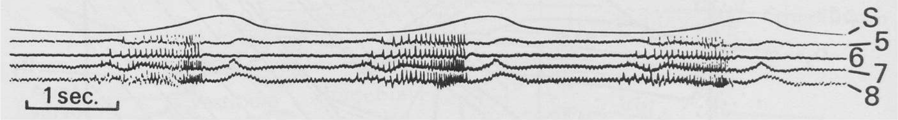

Fig. 4. *Anax.* Expiratory bursts recorded from the right respiratory dorso-ventral muscles of abdominal segments 5-8. Upper trace shows sternal movement (upwards indicates lifting). (From PICKARD & MILL, 1972).

Contraction of the respiratory dorso-ventral muscles lifts the abdominal sterna, thus causing an increase in pressure in the abdomen. This is transmitted to the branchial chamber and vestibule, where HUGHES & MILL (1966) have recorded pressures of 2-5 cmH_2O (Fig. 5).

During this time the anal valve is open to about a third of its maximum extent (MILL & PICKARD, 1972) (Fig. 6) and so the increased pressure causes the water in the branchial chamber to be ejected.

There are two abdominal inspiratory muscles, the diaphragm and the subintestinal transverse muscle (AMANS, 1889; WALLENGREN, 1914) and they are so arranged that, when they contract, they help to force the sterna down (TONNER, 1936). These muscles are not innervated by the segmental muscles, but by branches of the unpaired median nervous system (MILL, 1965). Their activity is synchronized and alternates with that of the expiratory muscles (MILL & HUGHES, 1966; MILL, 1970 (Fig. 7). The action of these muscles is assisted

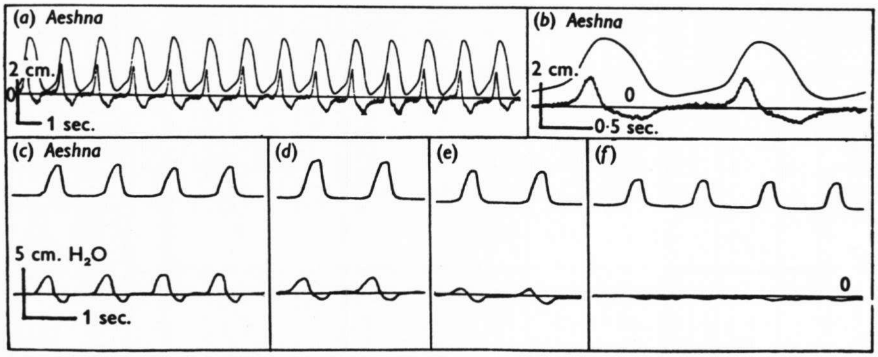

Fig. 5. *Aeshna.* Recordings of sternal movements (upper trace – upwards indicating lifting) and pressure in the branchial chamber with respect to the outside zero pressure (lower trace – upwards indicates increase in pressure). 0, zero pressure. (From HUGHES & MILL, 1966).

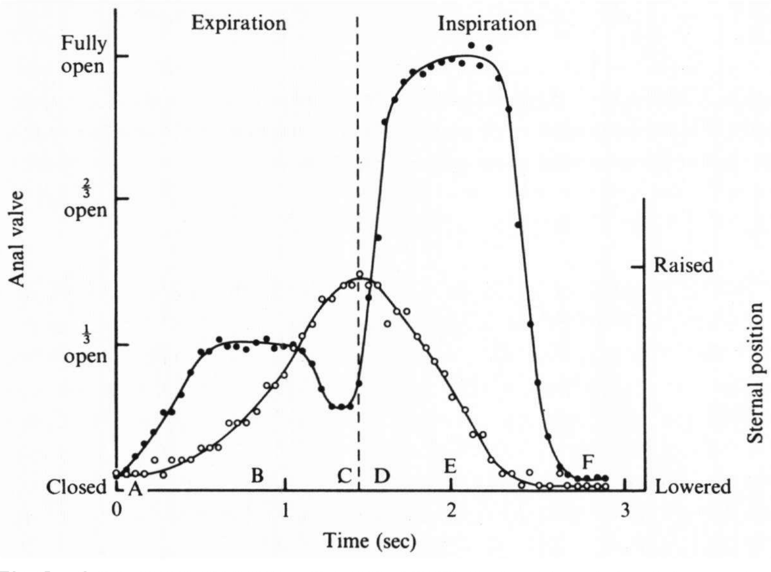

Fig. 6. *Anax.* Open anal valve area and sternal movement measured from projected ciné frames. (From MILL & PICKARD, 1972).

by the natural elasticity of the cuticle. Lowering of the sterna tends to create a negative pressure in the branchial chamber and vestibule and this is aided by contraction of the dilator muscles of the vestibule (TONNER, 1936). Negative pressures of ½-1 cm water have been recorded in the branchial chamber (HUGHES & MILL, 1966). During inspiration the anal valve is fully open and water is drawn in from close to the anus.

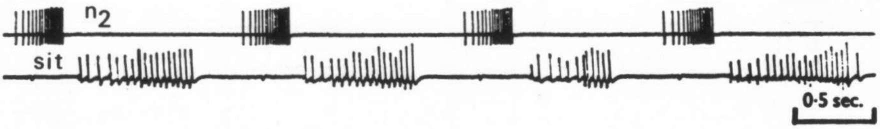

Fig. 7. *Aeshna*. Recording from a second segmental nerve of the seventh abdominal ganglion (n_2) (expiratory) and from the subintestinal transverse muscle (sit) (inspiratory). (From MILL, 1970).

There is some indication that, under certain conditions, other muscles are also involved in expiration. In dissected preparations bursts of action potentials have been recorded from all three pairs of segmental nerves coincident with those in the motor nerves to the respiratory dorso-ventral muscles, although no other muscle contractions were noticed (MILL, 1970) (cf. *Libellula* larvae; MILL, 1968). However, there is evidence for synchronous activity in the anterior dorso-ventral muscles in some instances in chronic preparations. This is comparatively unusual and may occur when the animal is under respiratory stress and needs to increase its rate of ventilation. The activity recorded in the first segmental nerves may be going to the longitudinal muscles to effect an increase in tension rather than an active contraction. If so this may be to prevent elongation of the abdomen during the increased pressures of expiration.

OTHER FORMS OF VENTILATION

There are various other forms of ventilation. In the "gulping" type described by TONNER (1936) the vestibule first fills with water and then the anal valve is closed, the post-branchial valve opened and the water forced into the branchial chamber by contraction of the vestibule. This is repeated several times, so "pumping up" the branchial chamber. The water may be then moved around in the branchial chamber ("chewing" of TONNER, 1936). Finally the anal valve opens and there is a slow expiratory phase.

In the current work two types of ventilatory activity have been seen to occur, other than normal ventilation. In one case the sterna are lifted and held in this position, presumably by the intermittent bursts of activity in the respiratory dorso-ventral muscles (HUGHES & MILL, 1966; PICKARD & MILL, 1972) (Fig. 8a, b). In the other type the sterna are initially raised, but then start to fall slowly towards their resting position. This fall is regularly interrupted by brief, rapid bursts of activity in the respiratory dorso-ventral muscles (Fig. 8c, d), and it is of interest to note that this activity decreases from posterior to anterior (PICKARD & MILL, 1972). However, there is still a great deal to learn about the various forms of prolonged ventilatory movements, and the two described in this paragraph may well be extreme examples of one type.

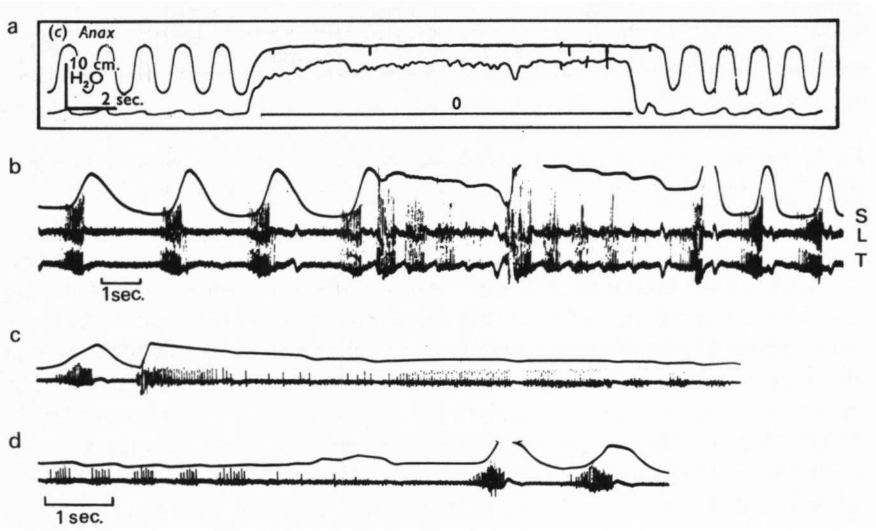

Fig. 8. ***Anax.*** **"Gulping" ventilation. Sternal movements are shown on the upper trace in all records (upwards indicates lifting): (a) recording of pressure in the branchial chamber with respect to the outside zero pressure (upwards indicates increase in pressure). 0, zero pressure. (b) recordings from the left (l) and right (r) respiratory dorso-ventral muscles of the sixth abdominal segment (lower trace). (c, d) recording from the right respiratory dorso-ventral muscle of the fifth abdominal segment (lower trace). [(a) from HUGHES & MILL, 1966; (b-d) from PICKARD & MILL, 1972].**

JET-PROPULSION

Essentially jet-propulsive activity appears to be a rapidly executed form of normal ventilation, with the additional external characteristics that the ab-

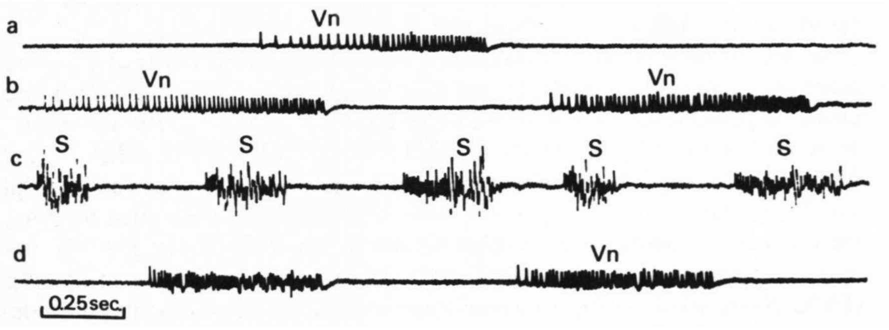

Fig. 9. ***Anax.*** **(a-d) Continuous recording from the left respiratory dorso-ventral muscle of the seventh abdominal segment during normal ventilation (Vn) and swimming (S).**

dominal segments are telescoped and the legs are moved backwards during the "expiratory" phase. However, additional muscles are involved in raising the sterna, including the anterior and posterior dorso-ventral muscles. Furthermore, the potentials recorded from these and from the respiratory dorso-ventral muscles are considerably larger than those recorded from the respiratory dorso-ventral muscles in normal ventilation (Figs. 9, 10). It is thought that the longitudinal telescoping is primarily effected by the longitudinal tergal and sternal muscles. TONNER (1936) suggested that the oblique musculature is also involved. If so this could have the dual effect of helping to lift the sterna and telescope the abdominal segments. The pressure in the branchial chamber normally exceeds 30 cm H_2O in both *Anax* (HUGHES, 1958) and *Aeshna* (HUGHES & MILL, 1966) (Fig. 11).

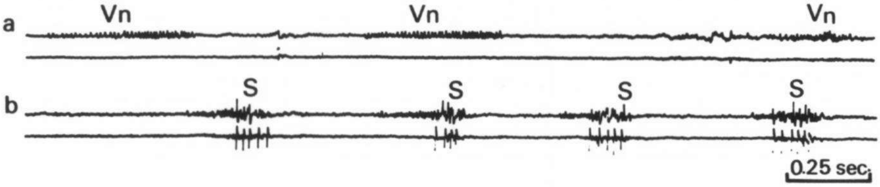

Fig. 10. *Anax*. (a-b) Continuous recordings from the right respiratory dorso-ventral muscle of the seventh abdominal segment (upper trace) and the left posterior dorso-ventral muscle of the seventh abdominal segment (lower trace) during normal ventilation (Vn) and swimming (S).

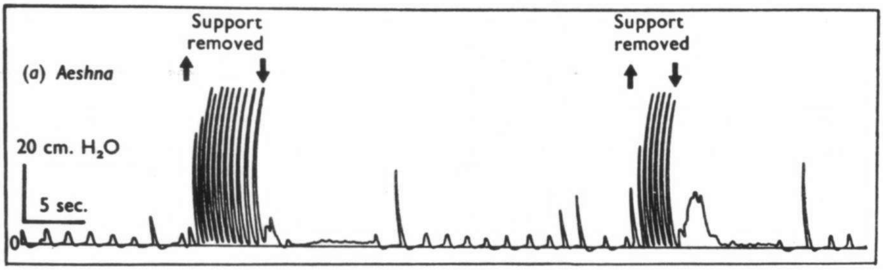

Fig. 11. *Aeshna*. Pen recording of pressure changes in the branchial chamber during normal ventilation (small, rhythmic changes) and swimming (large amplitude changes). (From HUGHES & MILL, 1966).

NATURE OF THE NORMAL RHYTHM

The ventilatory rhythm is a centrally produced pattern which can probably continue in the absence of any sensory input. However, its frequency can be adjusted by sensory input to the posterior abdominal segments. Thus, if the first segmental nerves are electrically stimulated between expiratory bursts an expiratory burst is immediately initiated and the rhythm reset (MILL & HUGHES, 1966). Repetitive bursts of stimuli delivered to a first segmental nerve at a frequency slightly greater than that of ventilation cause the rhythm to be "locked in" to this new frequency. The original frequency is immediately resumed on cessation of stimulation (MILL, 1970) (Fig. 12).

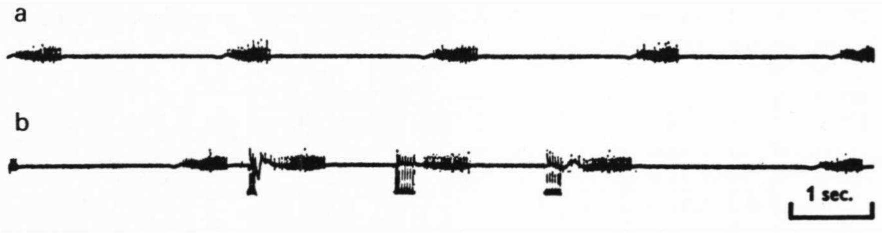

Fig. 12. *Anax*. Continuous recording from a second segmental nerve of the sixth abdominal ganglion. Repetitive electrical volleys to the contralateral first segmental nerve of the same ganglion are indicated by the lower marker. (From MILL, 1970).

It is suggested that "command" interneurones from the thorax drive an expiratory pacemaker in the last abdominal ganglion. There is presumably a positive feed-back loop which causes the motor output to increase in frequency, but when a certain level is reached the expiratory activity is cut off and inspiratory activity started (MILL & HUGHES, 1966). There is evidence that the sixth ganglion sometimes fires before the seventh, which indicates that the centre in the sixth ganglion at least receives an input from the "command" system. Successively more anterior ganglia may have higher thresholds and only be activated when the information from the "command" system is augmented by activity from the next most posterior ganglion (PICKARD & MILL, 1972). The general nature of the system is reminiscent of the rhythmic movements of crayfish swimmerets (HUGHES & WIERSMA, 1960; IKEDA & WIERSMA, 1964; WIERSMA & IKEDA, 1964).

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